EXPERIMENTS IN METASTASIS OF THE BROWN-PEARCE TUMOR IN RABBITS

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Part I

These experiments were designed to investigate various aspects of the problem of blood borne metastases of tumors. The tumor which was chosen to implement these investigations was the Brown-Pearce Rabbit tumor first described by Brown and Pearce in May 1923. (8) This tumor was chosen because much is known about its histology and other characteristics which have been observed during more than 30 years of study by several investigators.

A more detailed description of various aspects of the tumor and its earlier history will follow in another section. The tumor may be easily carried by the anterior chamber transplantation technique developed by Greene and this was the source of tumor material in these experiments.

It was decided to make an emulsion of tumor cells recovered from the anterior chamber of a rabbit's eye and to inoculate this emulsion into the ear vein of another rabbit. It was assumed that the method of introducing the tumor into the rabbit resembled the situation of blood borne metastases by tumor cell emboli, or that pertaining when tumor cells enter the circulation via the thoracic duct or other lymphatic channels. It was hoped that with this method, we could study one mode of metastases without the possibility of tumor spread by local lymphatics, direct extension or other means. Naturally, all tumor cells would be required to pass through the pulmonary vascular

bed before entering the left heart and it was assumed that the filtering action of this capillary bed would be fairly constant if a standard number of cells were injected and the emulsions did not contain large clumps of tumor cells. Smaller clumps, however, were present in all emulsions because they are evidently necessary for metastases. (24) From the left heart the tumor cells would be distributed to the various organ systems in approximate ratio to the blood flow at the moment of inoculation.

It might be mentioned at this time that there are certain very definite differences between this artificial intravenous tumor spread and the normal process of metastases, even of the blood borne embolic type.

1) The rabbits used had never been exposed to the Brown-Pearce tumor previously and, therefore, had no possible acquisition of "resistance", antibodies or "blood sweeping factors" except those inherently present. All of these might be theoretically present together with other unknown factors to influence metastasis in the "normal" rabbit with the tumor acquired by transplantation or other means.
2) These tumor cells were placed only into the ear veins in relatively close proximity to the right heart and pulmonary circuit and therefore may have a much wider distribution than embolic cells entering end arteries; e.g., mesenteric, splenic or gastric veins; or other specific areas of the circulation.

3) The entire number of tumor cell emboli were introduced at one instance whereas in the usual process of blood borne metastases, cells are probably periodically released into the systemic or local circulatory system.

4) The possibility of secondary metastases from various primary sites casued by the initial emulsion injection is undoubtedly small because of the methods used but can not be entirely discounted.

5) The size of the "emboli" was fairly constant (single cells and small clumps) in these experiments but might vary with the usual modes of metastases.

With these and possible other factors in mind it is obvious that the experimental conditions only approximate the usual development of metastases but at the same time the method minimizes the number of variables present and presents a quick and reliable method of producing metastasis as well as a definite standard or pattern from which deviations might be measured. A more detailed explanation of methodology will accompany the descriptions of the experiments.

It is planned to describe the general pattern of tumor growth in relation to size, sites, time, etc., and then investigate various factors which might alter this basic pattern of "metastases" in relation to these standards. The factors investigated by this writer included such variables as sex, hormonal, and metabolic factors. The progression and regression of the metastatic sites was also investigated. Various physical and physico-hormonal aspects

of the problem were studied by another investigator in the same laboratory.

Part II

Description Experiment I.

The tumor described by Brown and Pearce was first discovered in a scrotal chancre of a syphilitic rabbit four years after the inoculation of Treponema Pallidum. The initial tumor mass (2) was followed by diffuse pelvic and lower abdominal spread. Histologically the tumor was described as resembling surface epithelium with nests and whorles of cells and a vascular, strand like stroma. Invasion of local lymphatics with spread to regional nodes was also seen. Further observations noted metastatic lesions in the lungs, liver, kidney, spleen, bones and lymph (2) nodes. An interesting observation on the adrenals noted a decrease in the amount of cortical material.

Transplantation (3) of the tumor was most successful to organs of low capacity for granulomatous tissue response and inoculation of the testes was used as a means of carrying the tumor for further investigation. Metastases were noted to occur in 4 to 10 weeks after inoculation. Studies in the metastatic pattern of this tumor are recorded in Table 1.

Table I

Metastases from Testicular Inoculation Site

First lesions - 2 weeks: Maximum lesions - 7 weeks.

Kidneys	50%	incidence
Lungs	36%	"
Adrenals	32%	"
Lymph Nodes	25%	"
Liver	25%	"
Eyes	15%	"
Muscles	14%	"
Bones	13%	"
Heart	12%	"
Pituitary	11%	"
Skin	9%	"
GI tract		
Pancreas		
C.V. system		Rarely or never involved in metastases
C.N.S.		
Spleen		
Thyroid		

The authors (3) at this time did not feel that the spread of the tumor could be explained entirely by dissemination of tumor cells but believed that local tissue factors must play a part as well as constitutional aspects of the host. Pearce and Brown noted variations in the "resistance" of certain animals and organ systems but noticed a definite uniform distribution of metastases when they occurred. They noted that "growth rarely obtained with intravenous introduction of the tumor" which they believe argues for a blood stream factor which "clears" cells in transit. They described three important factors in explaining their results.

1. The constitutional state of the host.

a. Nutrition

b. "resistance" to tumor growth

2. Transport of tumor cells by the blood stream. Many cells disposed of in circulatory system. Increase in fibrin and thrombin formation secondary to antigen-antibody reaction.

3. Local tissue factors.

a. Mechanical-location in systemic circulation

b. Nutritional-local circulation, etc.

c. Inflammatory-granulomatous responses decrease tumor takes. Foreign body responses.

d. Hormonal-?

More recent study in the distribution of blood borne metastasis has been reported by Coman. (5) He argues that loss of cohesiveness and the assumption of ameboid motility by tumor cells are prerequisites to invasiveness and metastasis. The invading tumor cells following paths of "least resistance" enter lymphatics and veins and proliferate when they are lodged at various sites in the lymph nodes or systemic circulation. Coman feels that mechanical factors are most important in explaining sites of metastatic growth. The factors involved in the number of metastases according to Coman are:

1. Number of embolic cells - majority fail to grow.

2. Age of primary growth.

3. Size of primary tumor.

Increases in 1), 2), or 3) cause an increased number and rate of metastasis. In further work (5) Coman describes the staining of

Brown-Pearce tumor cells, their inoculation into the left ventricle and their recovery in the organs of most frequent tumor metastasis. He concludes that anatomical distribution of the tumor is related directly to the frequency of embolic cells passing to the specific organs.

Prinzmetal (16) has described work in which glass beads of greater diameter than the pulmonary capillaries were seen to pass through the pulmonary circulation indicating the presence of some sort of A-V shunting mechanism in the lung. This information is of special interest in our experiments because all of the tumor cells inoculated were required to pass through the pulmonary circuit before entrance to the systemic circulation and from the data obtained it seems likely that most of the injected cells or cell clumps passed through the lungs without lodgement although the question of primary growth in the lung with secondary tumor embolization cannot be entirely excluded.

Methods used in Experiment I.

This experiment was designed to serve as a standard of reference for succeeding experiments by this writer and for others working in the same laboratory as well as to provide information about certain variables to be considered in analyzing metastatic growth.

1) An emulsion was prepared with tumor recovered from the anterior chamber of a rabbit's eye. The tumor material was finely ground together with sterile saline agar broth

in a glass emulsifier until there were no large aggregates remaining.

2) Cell counts were made by the white blood cell counting chamber technique and the number of cells per mm (25) was maintained between 2300 and 5100 cells. (24) Previous work in this laboratory has shown that this variation is not significant providing small cell clumps are present.

3) One cc. injections of tumor cell emulsion were made into the ear vein of rabbits on one occasion for each rabbit as noted in Table II.

4) The rabbits were maintained on Standard Purine Rabbit food.

5) The rabbits were killed at varying time intervals from 13-34 days after inoculation.

6) Autopsies were performed on all rabbits with examination grossly of all major organ systems. Microscopic sections were taken from various portions of all major organs in these animals and examination made for microscopic tumor emboli which may have escaped notice grossly.

7) Only New Zealand white rabbits were used in this experiment. Eight male and sexteen female rabbits were used. This experiment was performed during the summer months of July and August and generally in air conditioned surrondings. It was expected that previously noted

seasonal variations in the tumor characteristics were kept at a minimum.

The following table describes the procedures performed on each rabbit.

Table II

No.	Sex	Date injected	No. of cells mm3	Date of Autopsy	No. of Days
1	F	July 6	2875	July 19	13
2	F	July 6	2875	July 22	16
3	F	July 6	2875	July 27	21
4	F	July 6	2875	July 27	21
5	F	July 7	2375	Aug 1	26
6	F	July 7	2375	Aug 1	26
7	F	July 7	2375	Aug 1	26
8	M	July 7	2375	Aug 1	26
9	F	July 7	2375	Aug 5	30
10	F	July 7	2375	Aug 5	30
11	F	July 7	2375	Aug 5	30
12	F	July 7	2375	Aug 5	30
13	M	July 7	2375	Aug 9	34
14	F	July 7	2375	Aug 9	34
15	F	July 7	2375	Aug 9	34
16	M	July 7	2375	Aug 9	34
1'	M	Aug 5	5100	Aug 29	24
2'	M	Aug 5	5100	Aug 29	24
3'	M	Aug 5	5100	Aug 29	24
4'	M	Aug 5	5100	Aug 29	24
5'	F	Aug 5	5100	Aug 29	24
6'	M	Aug 5	5100	Aug 29	24
7'	F	Aug 5	5100	Aug 29	24
8'	F	Aug 5	5100	Aug 29	24

Results of Experiment I

The data obtained can be best presented in chart form (Table III). The analysis of this data centered about the following items.

1. Time interval and location of first metastasis.
2. Most common sites for metastatic growth.
3. Progression and regression in size and number of tumor sites.
4. Comparison of male and female in relation to frequency and site of metastatic growth.
5. Incidence of microscopic metastasis not grossly evident.

The results obtained:

1. The first metastases were seen sixteen days after tumor inoculation, these growths were microscopic and not in evidence by gross examination. The organs involved were the adrenals, kidneys, and ovary. No evidence of tumor growth was seen at thirteen days.
2. The following table records the data relevant to the organ systems commonly involved in tumor spread.

Table IV

Organ	20 animals	% involvement
Adrenal	18/20	90%
Kidney	15/20	75%
Ovary	8|12	66%
Eye	11/20	55%

* excluding four females with no metastasis

Table III

√ = gross metastasis
* = microscopic metastasis

Days	13	16	21	21	24	24	24	24	24	24	24	24	26	26	26	26	30	30	30	30	34	34	34	34
R. Adrenal			* √	* √	* √	* √	* √	* √	* √	* √	* √	* √				* √			*	* √	* √		* √	
L. Adrenal		*	* √		* √	* √	* √	* √	* √	* √	√	* √				* √	* √	*		* √				
R. Kidney		*	*	*			* √	*	* √	√	* √	*				* √				* √	* √	* √	* √	
L. Kidney		*					* √	*	* √	√	* √					* √		*		√	√	* √	* √	
R. Eye			*	*												* √							* √	* √
L. Eye			*				* √		* √	*	√					√		*		* √				
R. Ovary			* √	* √					* √								* √							
L. Ovary		*	* √	*					* √		√	* √										*		
Liver																								
Lungs																								
Heart																								
Spleen																								

√ = gross metastasis * = microscopic metastasis

Microscopic or macroscopic evidence of tumor involvement of the liver, lungs, heart, spleen, testes and other organs was entirely lacking in this experiment. Involvement of the spleen was noted in one rabbit by another worker in the same laboratory using similar techniques. Nodules closely resembling metastatic tumor were frequently found in the liver but microscopic study revealed these universily to be biliary helminthic disease with flukes resembling Fasciola Hepatica.

3. The size of the growths increased and on the 21st day after inoculation first became grossly visible in the ovary and adrenal. The size and number of tumor sites progressively increased from the 24th to the 34th day after inoculation with some indication of regression about the 34th day.

4. The following table records data relevant to sex differentiation in the behavior of the tumor. (Table V)

5. A total of 79 separate organs were involved with tumor in this experiment. Of this number, 70 lesions were proven histologically with the proof resting on gross evidence in only nine cases (failure of microscopic evidence in these cases was probably due to inadequate tissue sectioning). Gross metastases were in evidence in only 60 different organs and therefore microscopic evidence had to be relied upon in 19 situations where

Table V

Location of Metastases

(Based on Microscopic evidence)

24 animals - 8 males, 16 females

R. Adrenal - 15		28
L. Adrenal - 13		
R. Kidney - 13		22
L. Kidney - 9		
R. Eye - 5		11
L. Eye - 6		
R. Ovary - 4	16 animals	10
L. Ovary - 6	16 animals	
Total Right = 37	Male = 24/8 = average of 3 metastasis / animal	
Total Left = 34	Female = 47/16 = average of 3 metastasis / " *	

* when corrected for 4 females with no metastasis 47/12 = Females

Female - 47/12 = 4 metastasis/animal

There is no significant difference noted in the behavior of the tumor in males and females in regard to tumor sites, speed of growth or lag period before the appearance of tumor growth. The ovary was involved with tumor growth in 66% of the females with demonstrable lesions of any sort but the testes were not involved in any male rabbit. Whether this differentiation is caused by anatomical, hormonal or possibly thermal conditions is not evident from these results.

the size of the tumor prevented macroscopic identification.

Discussion

This data relevant to the behavior of the Brown-Pearce tumor under fairly well standardized conditions was compiled and analyzed with the anticipation that it might be used as a control group. This relatively easy method of studying tumor spread by the vascular system is well adapted to the investigation of mechanical, hormonal or metabolic factors and their effect on metastatic tumor growth, sites of metastatic growth and methods of blood transport via the vascular system.

Experiment II

The second experiment was designed to show the influence of a specific hormone on the pattern and chronology of metastases described in Experiment I. Cortisone was chosen as a hormone which has been demonstrated in the past by Pomeroy and also by Duran-Reynals to vary both the rate of growth of certain tumors and the sites of metastases. (15) Widespread tumor metastases were rapidly seen in the Krebs II carcinoma in mice with high dosage of cortisone. However, cortisone had no effect on mouse sarcoma growth. (15) Cortisone has also been described as slowing the growth of the primary tumor site while favoring the development of metastatic lesions. Other evidence favors the (9)

belief that cortisone enhances the transplantability of tumors probably through its effects on immune mechanisms because it has an actual suppressive effect on the development of stroma by the transplanted tumor.

Method

Six rabbits were used in this experiment. They were given cortisone in doses of 5 mg. for two days before inoculation with Brown-Pearce tumor and five days following. Tumor emulsions and injections were identical with that of Experiment I.

Table VI

Date Aug	i.v. 15	16	i.m. 17	i.m. 18*	i.m. 19	i.m. 20	i.m. 21	i.m. 22	Killed at
1	X		X	X	X	X	X	X	20 days
2	X		X	X	X	X	X	X	21 days
3	X		X	X	X	X	X	X	21 days
4	X		X	X	X	X	X	X	21 days
5	X		X	X	X	X	X	X	21 days
6	X		X	X	X	X	X	X	23 days

* Brown-Pearce tumor emulsion injected intravenously.

The rabbits were killed at 20-23 days after tumor inoculation.

Results

Widespread metastases were found in 4 of the six animals. These were large and easily identified grossly whereas control rabbits (Experiment I) had only microscopic or barely visible tumor growth at these time intervals.

Table VII

	Adrenals	Kidney	Eye
20 day	R. and L.	R. and L.	no
21 day	no	no	no
21 day	R. and L.	R.	no
21 day	no	no	no
21 day	R. and L.	R. and L.	no
23 day	R. and L.	R. and L.	R.

An analysis of the results in the four rabbits which showed metastases and a comparison with untreated animals in Experiment I receiving similar preparations of tumor revealed:

1. Significantly larger and more dramatic gross tumor specimens in the cortisone treated rabbits as compared with untreated rabbits killed at 24 days.
2. Gross involvement of eye and kidney in the cortisone treated group which was not noted until 24 days in the control group. (Although microscopic evidence was present at 21 days in the controls.)
3. The slightly earlier (21 days) appearance of obvious gross metastases in the cortisone treated group as compared to the controls (24 days) is not considered significant although when considered in view of the distinctly larger size of the individual metastatic growths this timing may be considered suggestive of cortisone exhibiting an enhancing effect on "metastatic" tumor spread and growth.

Discussion

The most interesting aspect of this experiment is that it suggests that metastatic tumor growth which are present in animals carried on supplementary cortisone treatment may actually grow faster in size and with less "restraint" within the particular organ in which it is implanted. The experiment indicates that high levels of cortisone do not seem to alter the sites of metastatic growth and therefore seem to have little effect on the initial stage of metastasis. Since cortisone probably decreases the tendency toward stroma formation or fibroblast proliferation in areas surronding tumor (9) the most likely explanation for the suggestable effects of cortisone noted above lies in the area of inhibition of host resistance either by suppression of immune mechanisms or prevention of the effect of antigen-antibody reaction upon the host or tumor tissue. This experiment can not be considered in any way as proof of this hypothesis although it is certainly compatible with such an explanation.

Experiment III

There have been many reports in the literature about the importance of Vitamin C metabolism in relation to tumor growth,(19) transplantibility, (12) and metabolism of tumor cells. (4) In view of the results of our previous experiments in which the adrenals were the most common and earliest site for "metastases" to

occur and of the interesting observations of Greene (8) that this tumor may be heterologously transplanted only to animals which endougenously manufacture Vitamin C (mice, rats, hamsters) it was felt that further investigations of the relation of Vitamin C to the growth patterns of this tumor were indicated. Vitamin C has been considered important in tumor growth for many years, various explanations being offered for such activity include:

1. Action on vascular system with enhancement of stroma development.
2. Involvement in important metabolic equations in the tumor cells, e.g., conversion of RNA to DNA. (19)

It has been noted in various experiments that Vitamin C may inhibit tumor growth as well as enhance it and also that the effect of tumor on the ascorbic acid levels of individual organs varies in relation to tumor type, animal and specific organ. (4) Brown-Pearce tumor transplants have been noted not to effect organ ascorbic acid levels in the DBA mouse while other tumors increase these levels. (11) Since the rabbit is an animal which manufactures Vitamin C and the Brown-Pearce tumor appears to require such circumstances for growth, an experiment was designed in which animals were treated with high doses of ascorbic acid and then given intravenous tumor cells by our standard technique and observations were made on the rate of growth and distribution of the tumor.

Method

Five female New Zealand rabbits were used in the experiment and one additional animal was used as a control. The five animals received doses of ascorbic acid in amounts of 100 mg. for three days prior to tumor inoculation. They received 200 mg. on the day of injection and 100 mg. on each of five succeeding days. One animal was killed at 13 days, the remainder and the control were killed at 14 days. The tumor emulsion was prepared in the usual way and 1 cc. was injected into the ear vein of each rabbit. The control rabbit received no ascorbic acid. At autopsy gross tumor sites were noted as well as relative size of the tumor growths. Microscopic sections were also taken of suspicious small lesions and of the adrenals and kidneys in every case. The time interval of 14 days was chosen because it is a full week earlier than gross tumor metastases had been noted before and it was felt that a 30% decrease in time necessary for gross tumor appearance would be definitely significant.

Results.

Five animals with 1100 mg. of ascorbic acid.

Table VIII

Adrenal	Both	Both	One	Both	No	No
Kidney	One	One	Both	Both	One	No
Ovary	One	No	No	No	No	No
Eye	No	One	No	No	No	No
Liver	No	No	No	One	One	No
Lung	No	No	No	No	One	No
Other	No	No	No	No	No	No
	1	2	3	4	5*	Control

* 13th day

Table IX

	% tumor involvement
Adrenal	80%
Kidney	60%
Liver	40%
Ovary	20%
Lung	20%
Eye	20%

It was noted that all five animals treated with high doses of ascorbic acid showed tumor growth in various organs by 14 days. The control animal showed no growth and the results of Experiment I indicate that gross metastases do not occur before 21 days and are not prominant until 24 days. It is of further interest that these various tumor implantations were relatively large and easily seen grossly except in the one case of lung involvement which was seen microscopically only. It is logical to assume that these tumor growth were actually visible before 14 days and may have been microscopically evident as early as 10-12 days. A comparison of the results with those of Experiment I is represented in the following Table X.

Table X

	Controls	Ascorbic Acid
1st Gross Tumor	21 days	13 days
Size of 1st Gross Tumor	very small	much larger
Number of "metastases" per animal	3	4
Liver, Lung involvement	no	yes

Discussion

The results of this experiment pose many questions for interpretation. Among them:

1. The most obvious question posed is why and by what mechanism does ascorbic acid seem to enhance the rapid growth of the Brown-Pearce tumor when it is injected by the standard intravenous method.
2. Another problem is the explanation of the involvement of liver and lung in a few animals of this series.
3. An explanation for the consistent involvement of adrenals and kidneys with or without ascorbic acid supplement should be sought.
4. The relationship of these results to the previously noted (8) observation that the Brown-Pearce tumor can not be transplanted to the Guinea Pig, an animal with the human variety of Vitamin C metabolism.
5. How is this related to the high tissue ascorbic acid levels noted in animals with transplants of certain tumors (but not Brown-Pearce.)
6. Can these results be compared to the noted increase utilization of Vitamin C by human patients with metastatic disease. (23)

I will attempt to discuss possible answers or interpretations of these questions in the light of the observations of these

experiments and those of others.

1. Others have noted in the past (19) that low levels of Vitamin C produced by scorbutic diets and d glucoascorbic acid (an ascorbic acid inhibitor) would retard tumor growth. The explanation previously offered is that Vitamin C is essential for the rapid conversion of RNA to DNA in the cell. These experiments did not prove that high doses of Vitamin C and resulting high DNA values increased the rate of tumor growth. It seems that the explanation most likely for the observations of our experiment lie in the area of tumor cell metabolism because the individual tumor sites appeared earlier and were larger although there did not seem to be any more widespread tumor involvement in these animals. Since nucleic acids are essential for rapidly proliferating cells the RNA to DNA mechanism of Sokoloff may be one explanation for these observations. (19) The implication involved here is that tumor cells multiply at a maximum rate when large levels of ascorbic acid are present and other factors are constant because they are able to produce nucleic acids at a maximal rate. It is also of interest that an increase utilization of Vitamin C by humans with metastatic disease has been observed. (23)
2. The tumor noted in the lung in one animal was a small microscopic area and it is possible that this represented an embolic tumor cell clump possibly with some proliferation.

It is possible that all of our animals might show such lesions early but that in the control group the period of 24 days is long enough for resorption or death of these microscopic areas in the lung which has a well known poor stroma producing capacity. (5) It is also possible that high ascorbic acid levels enabled this tumor to grow even in the poor "soil" of the lung. The large liver growths probably are related to the latter explanation.

3. The adrenals and kidneys are persistently involved with tumor although one animal in this group had normal adrenals but did have kidney "metastases". The adrenals especially but also the kidneys are organs with a well known high level of ascorbic acid. The frequent involvement of these organs may be explained on the basis of their rich blood supply, adequate stroma producing capacity and possibly their high ascorbic acid levels. This does not explain the relative infrequency of brain and splenic involvement, both areas of high ascorbic acid content.

4. It is likely that the Brown-Pearce tumor is dependent upon a certain tissue or blood level of ascorbic acid for rapid growth. Guinea Pigs which rely on exogenous Vitamin C are poor hosts for the tumor. Our experiments are one more hint of the importance of ascorbic acid in

the metabolism of this tumor.

5. In animals who produce endogenous Vitamin C it is likely that certain tumors may induce the production of higher levels of ascorbic acid. Our data seem to indicate that Brown-Pearce tumor growth is enhanced by high doses of Vitamin C although this tumor was not one noted to produce increase tissue levels of this vitamin in the experiment of Leise and Harvey. (11)

6 and 7 These questions, I believe, are open to further fruitful investigation. Vitamin C plays some part in the rate of growth of certain tumors. Such tumors should be delineated and the mechanism of this action, whether biochemical on the tumor cells or specific on the animal tissue involved, ought to be investigated. It seems possible from past results (23) and from our experiments that metastatic tumors require and utilize large amounts of Vitamin C and this might be used as a diagnostic tool for such metastatic growth.

Summary

Essentially three experiments were performed. The first was intended as a control and involved only the intravenous introduction of tumor into rabbits and the delineation of the sites and time intervals necessary for growth to appear. Specific results are noted in the tables.

Experiment II investigated the effect of cortisone given before and after tumor injection upon the patterns elicited in the first experiment. The results suggested some small enhancement of growth of the tumor.

Experiment III observed the dramatic effects of Vitamin C upon the standard pattern first seen. Gross tumor sites appeared 30-40% earlier than in the control group and growth occurred in the liver and lung, areas not previously the site of tumor growth. An attempt at possible explanation of this phenomenon is made.

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